

Genetic Analysis of Meiotic Recombination in Humans by Use of Sperm Typing: Reduced Recombination within a Heterozygous Paracentric Inversion of Chromosome 9q32-q34.3

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Summary

To investigate patterns of genetic recombination within a heterozygous paracentric inversion of chromosome 9 (46XY inv[9] [q32q34.3]), we performed sperm typing using a series of polymorphic microsatellite markers spanning the inversion region. For comparison, two donors with cytogenetically normal chromosomes 9, one of whom was heterozygous for a pericentric chromosome 2 inversion (46XY inv[2] [p11q13]), were also tested. Linkage analysis was performed by use of the multilocus linkage-analysis program SPERM, and also CRI-MAP, which was adapted for sperm-typing data. Analysis of the controls generated a marker order in agreement with previously published data and revealed no significant interchromosomal effects of the inv(2) on recombination on chromosome 9. FISH employing cos-mids containing appropriate chromosome 9 markers was used to localize the inversion breakpoint of inv(9). Analysis of inv(9) sperm was performed by use of a set of microsatellite markers that mapped centromeric to, telomeric to, and within the inversion breakpoints. Three distinct patterns of recombination across the region were observed. Proximal to the centromeric breakpoint, recombination was similar to normal levels. Distal to the telomeric breakpoint, there was an increase in recombination found in the inversion patient. Finally, within the inversion, recombination was dramatically reduced, but several apparent double recombinants were found. A putative model explaining these data is proposed.

Introduction

Paracentric inversions are one of the most common structural chromosomal polymorphisms in humans. Most individuals carrying an inversion have a normal phenotype, but there are associated clinical manifestations including fertility problems, repeated spontaneous abortions, mental and congenital abnormalities, and an unbalanced karyotype of progeny (see Madan 1995 for a review). The risk that progeny from an inversion heterozygote may have chromosomal imbalance is dependent on the nature of meiotic crossing over within the inversion. Except for family studies, information on recombination across inversions in humans has been limited to the sperm karyotyping of male carriers (Martin 1986; Colls et al. 1997). In a series of cases, Hulten and colleagues demonstrated substantial changes of chiasma formation within the interstitial segments of human male translocation heterozygotes (Laurie et al. 1984; Goldman and Hulten 1993a, 1993b). Recently, in an attempt to study genetic recombination patterns around the breakpoints of autosomal balanced translocations, He et al. (1996) genotyped families for 20 polymorphic markers within 10 cM of chromosomal breakpoints and concluded that, despite the use of large families, there were not enough meioses to allow meaningful conclusions from the data. This problem can be overcome with the technique of sperm typing (Li et al. 1988; Cui et al. 1989), which allows the study of a potentially unlimited number of meioses. High-resolution genetic maps of regions of chromosomes can be constructed, thereby realizing the potential for studying variability of recombination both between and within chromosomes. Sperm typing has been used in a number of wide-ranging recent reports including the ordering of closely linked markers on chromosome 9 (Furlong et al. 1993); studies of trinucleotide repeat mutations in Kennedy disease (Zhang et al. 1995), Huntington disease (Leefflang et al. 1995), and Machado-Joseph disease (Takiyama et al. 1997); construction of a high-resolution multipoint linkage map of the human pseudoautosomal region (Schmitt et al.

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1994); analysis of meiotic drive at the myotonic dystrophy locus (Leeftang et al. 1996); and analysis of heterogeneity of recombination between human males (Yu et al. 1996). This study reports the pattern of genetic recombination found across a paracentric inversion of chromosome 9 by PCR typing of single sperm cells from a carrier with the karyotype 46XY inv(9) (q32q34.3), employing a set of polymorphic microsatellite markers spanning the region. For comparison, sperm-typing data are presented from two other individuals with cytogenetically normal chromosomes 9—one with a normal karyotype and another heterozygous for a chromosome 2 pericentric inversion (46XY inv[2] [p11q13])—to check for nonspecific effects of chromosomal inversion. Comparison of these donors' results allows investigation of interindividual differences in recombination, which has important implications, not only in the production of reliable, high-resolution genetic maps, but also for genetic counseling.

Methods

Sperm Donors

Donor Sp9, an anonymous 36-year-old man with a normal karyotype, was obtained from Addenbrooke's Hospital NHS Trust Cytogenetics Laboratory. Donor inv(2), who is cytogenetically normal for chromosome 9 but is heterozygous for a pericentric inversion of chromosome 2 (46XY inv[2] [p11q13]), and donor inv(9) were ascertained by the Regional Genetic Laboratory Services, Birmingham Heartland Hospital NHS Trust. Donor inv(9) is heterozygous for a paracentric inversion on the long arm of chromosome 9 (46XY inv[9] [q32q34.3]). His wife, who had suffered multiple miscarriages, is karyotypically normal. He has had two children, one of whom is confirmed to carry the inversion. The patient's brother is also a carrier of the same inversion.

Sperm-Typing Protocol

Sperm cells were purified from seminal fluid by use of a 25% Percoll (Pharmacia) density gradient and were then flow-sorted into 96-well polycarbonate microplates (Techne) by use of the protocol described by Furlong et al. (1993). Immediately after sorting into wells, single sperm were lysed by the addition of 2 μ M 200 mM potassium hydroxide/50 mM dithiothreitol and 40 μ M mineral oil and were incubated at 65°C for 10 min. The cells were then neutralized by addition of 2 μ M 900 mM Tris-HCl pH 8.3/300 mM KCl/200 mM HCl and were spun at 1,000 g to mix. Sperm cells were amplified by use of two rounds of PCR. First, a modified PCR protocol, based on the primer-extension preamplification (PEP) method of Zhang et al. (1992), was used to am-

plify the genome of each cell. Each cell was amplified in a 20- μ M reaction containing 100 mM Tris-HCl, 50 mM KCl, 2.5 mM MgCl₂, 200 μ M each dNTP, 0.01% gelatin, 400 μ M random 15-base oligonucleotide, and 5 U *Taq* polymerase (Cetus). PCR was performed by use of a Techne PHC 3 cyclor as follows: 95°C for 4 min; 50 cycles of 94°C for 1 min, 37°C for 2 min, and then ramping at 10 s/degree to 55°C for 4 min; and a final extension of 72°C for 10 min. The second, locus-specific round of PCR involved amplification of aliquots of the first-round product in an isotopic reaction: Typically, for each marker, 1 μ l of PEP product was amplified in 25 mM N-tris(hydroxymethyl)methyl-3-aminopropanesulfonic acid, pH 9.3; 50 mM KCl; 1–2 mM MgCl₂ (determined empirically for each set of primers); 40 μ M each primer; 1 mCi α [³²P]-dCTP; 125 μ M each of dATP, dGTP, and dTTP; 50 μ M dCTP; 0.1 U Perfect Match (Stratagene); and 0.5 U *Taq* polymerase (NBS). PCR was performed on a Techne PHC3 cyclor as follows: 95°C for 4.5 min; 55 cycles of 94°C for 30 s, 50°–60°C (depending on the melting temperature of primers in the reaction) for 30 s, and 72°C for 30 s; and a final extension of 72°C for 10 min. PCR products were resolved by electrophoresis on a 6% denaturing polyacrylamide gel at 50 W for ~3 h and were then visualized by autoradiography. Often, multiplexes of two or three pairs of primers were amplified in the same reactions. To aid scoring of alleles, the products of amplification of 20 cells were included on each gel as a reference.

Observer Bias and Error Checking

In an attempt to eliminate observer bias, autoradiograms were scored randomly so as to avoid phase expectations of the scorer. The haplotypes obtained from the raw data were run through the "chrompic" option of CRI-MAP (see below). This demonstrated the phase relations of each marker in each informative sperm and revealed any crossovers that were unsupported by flanking markers. Initially, for each sperm identified by the program, the autoradiogram results were rechecked against the scoring entered into the computer data file. Discrepancies in which the allele displayed on the autoradiogram did not match that entered into input file were corrected and the data included. If the entry in the computer file matched the autoradiogram score, then for a single recombinant chromosome, the result was assumed to be correct and no retyping was carried out. Since double crossovers in the region studied are likely to be rare, and triple and quadruple chromosomes are highly unlikely, sperm with two or more apparent crossovers were retyped for all of the loci.

Linkage Data Analysis

For initial data analysis, CRI-MAP version 2.4 (Lander and Green 1987) was adapted for the analysis of sperm-typing data (as described by Furlong et al. 1993). Maximum likelihood estimates from the sperm-typing data were performed with the program SPERM (developed from MENDEL; Lange et al. 1988), which calculates θ (and corresponding confidence intervals) for adjacent loci. Different models, specifying different parameters to be considered in the calculations, were tested for the data. Parameters included the method of amplification (i.e., direct PCR of loci or use of the PEP method) zero, one, or two cells amplified in a well; and allele-specific, locus-specific, and nonspecific amplification and contamination rates. Data were analyzed with all models and the results compared by use of likelihood ratio criteria. From these analyses, and following the principles of parsimony, a model was chosen and applied to the data of each of the three donors. For a detailed account of the software see Lazzeroni et al. (1994).

Statistical Analysis

Comparison of recombination fractions between donors Sp9 and inv(2) for a given marker interval by use of SPERM was performed by means of the following general equation. To test, $\theta_{12}1$ (loci 1 and 2, donor 1) = $\theta_{12}2$ (loci 1 and 2, donor 2),

$$\text{refer } \frac{\theta_{12}1 - \theta_{12}2}{[(SE1)^2 + (SE2)^2]^{1/2}} \text{ to } N(0,1),$$

where SE indicates standard error.

FISH Analysis

Metaphase spreads of inv(9) chromosomes were hybridized with pairs of cosmids, which contain the appropriate loci, each labeled to give a different fluorescent signal. The two-color FISH protocol is described in detail by Leversha (1997). Cosmids that contain D9S170 were not available; instead, cosmids containing ORM (9q32), which maps centromeric to D9S170, and ALAD (9q33), which maps very close to D9S170, were used. FISH was performed by use of cosmids containing the markers ORM, ALAD (Harris et al. 1993), D9S58, D9S59, D9S66, D9S67 (Kwiatkowski et al. 1992), D9S125, and D9S149 (Zhou et al. 1995).

Results

Analysis of Genetic Recombination of Control Donors Sp9 and inv(2)

Both donors, who were cytogenetically normal for chromosome 9, were initially screened by use of a panel

of >20 microsatellite markers spanning 9q31–9q34.3. Of these, a subset of 10 markers (ASS, D9S59, D9S66, D9S67, D9S109, D9S125, D9S149, D9S154, D9S170, and D9S315) was used to generate linkage data. Marker selection for each donor was based on both donors exhibiting heterozygosity for each marker with well-spaced alleles to aid scoring and a well-defined map position (Povey et al. 1996). For donor Sp9, by employing eight markers (ASS, D9S59, D9S66, D9S67, D9S109, D9S149, D9S154, and D9S315), 404 sperm were found to be informative for more than two loci, with >90% of cells informative for five or more loci. The most efficient loci, D9S315 and D9S149, were amplified in 91% of cells, and the least informative marker, D9S67, was present in ~70% of cells. The overall efficiency of locus amplification calculated by SPERM was 82.6% (SE \pm 0.7%), and the overall nonspecific contamination rate was 0.6% (SE \pm 0.2%). Ninety-four percent of wells were estimated by the program to contain only one sperm, with no wells estimated to contain more than one sperm. Four sperm were found to have multiple recombinant events and were retyped. One of these sperm exhibited two double crossovers and was considered to be a result of more than one sperm in the well, contamination, or mutation during PCR and was excluded from the results. The other double recombinant sperm involved relatively large genetic distances and were included in the final dataset. The order of D9S109–D9S59–D9S154–D9S315–D9S149–ASS–D9S66–D9S67 was established for Sp9 with a maximum log likelihood of –207.8. This order agrees with previously published data (Povey et al. 1996). The odds against second best order in which D9S149 and ASS were reversed were 1.3×10^9 .

Donor inv(2) was informative for nine microsatellite markers. Seven of these (ASS, D9S59, D9S67, D9S109, D9S149, D9S154, and D9S315) were included in the Sp9 marker panel. For this donor, the markers D9S125 and D9S170 were added, and D9S66 was excluded, since this marker was uninformative. Four hundred and eight cells were informative for two or more markers, and 82% of cells typed gave information at five or more loci. There was a nonspecific contamination rate of 0.8% (SE \pm 0.2%). The order (order 1) of D9S109–D9S59–D9S170–D9S154–D9S315–D9S149–D9S125–ASS–D9S67 was established for donor inv(2), with a maximum log likelihood of –210.2. The log odds of the second order, which has a reversal of the closely linked markers D9S170 and D9S154, against order 1 were 0.133 (i.e., 1.4:1 against). The third most likely order was one in which D9S125 and D9S149 are reversed in order 1. The odds against this order were 3.3×10^4 , and the odds against all other orders were $>10^5$.

Data from the typing of CEPH families were kindly

provided by Jon Attwood (see Attwood et al. 1994) for the markers ASS, D9S66, D9S67, D9S109, D9S154, and D9S170, and the map order and genetic distances were established by use of CRI-MAP. The overall genetic male-specific map length of the CEPH data, from D9S109 to D9S67, was 43.8 cM (for six markers). The results of Sp9 compared favorably with the CEPH data; an overall length calculated by CRI-MAP for all eight markers was 39.4 cM, whereas the map length of inv(2), was 51.1 cM by use of data from nine markers.

As the map lengths of Sp9 and inv(2) were appreciably different, a comparison of variation of θ values between these donors was carried out by use of both CRI-MAP and SPERM. By means of CRI-MAP, the number of informative and recombinant chromosomes for each interval were established for both donors, and χ^2 tests were performed. There were no significant differences ($P > .05$ in all cases) in the recombination fractions of inv(2) and Sp9 in the six marker intervals (D9S109–D9S59, D9S59–D9S154, D9S154–D9S315, D9S315–ASS, ASS–D9S149, D9S149–D9S67) common to both donors. Similarly, by means of SPERM, no significant differences ($P > .05$ in all cases) were found between the recombination fractions for any of the intervals in common.

Donor inv(9)

Donor inv(9), an individual heterozygous for a paracentric inversion (9q32–q34.3), was analyzed by use of a set of microsatellite markers that mapped centromeric to, telomeric to, and within the inversion breakpoints. Donor inv(9) was heterozygous for ASS, D9S66, D9S67, D9S109, D9S149, D9S170, and D9S315, of the markers already used in the study. Donor inv(9) was uninformative for D9S59, so this was replaced with the marker D9S58, which lies ~1 cM centromeric to D9S59 on a male-specific map prepared by pedigree analysis (Kwiatkowski et al. 1992). Two-color FISH that used cosmids containing appropriate chromosome markers was used to localize the inversion breakpoint of inv(9). The analysis confirmed that the centromeric breakpoint was distal to D9S58 and proximal to D9S170 and that the telomeric breakpoint was distal to D9S149 and proximal to D9S66 (data not shown).

A total of 282 inv(9) sperm were typed. Two hundred and seventy sperm were informative for two or more markers, and 86% of sperm were informative for five or more markers. By means of SPERM, a nonspecific contamination rate of 0.006% (SE \pm 0.002%) was estimated, with 99.2% of wells estimated to contain a single sperm. After error checking and retyping, six chromosomes were shown to be multirecombinant, with five of these having recombination events within the inversion. Figure 1 shows typing of these sperm. Sperm a was

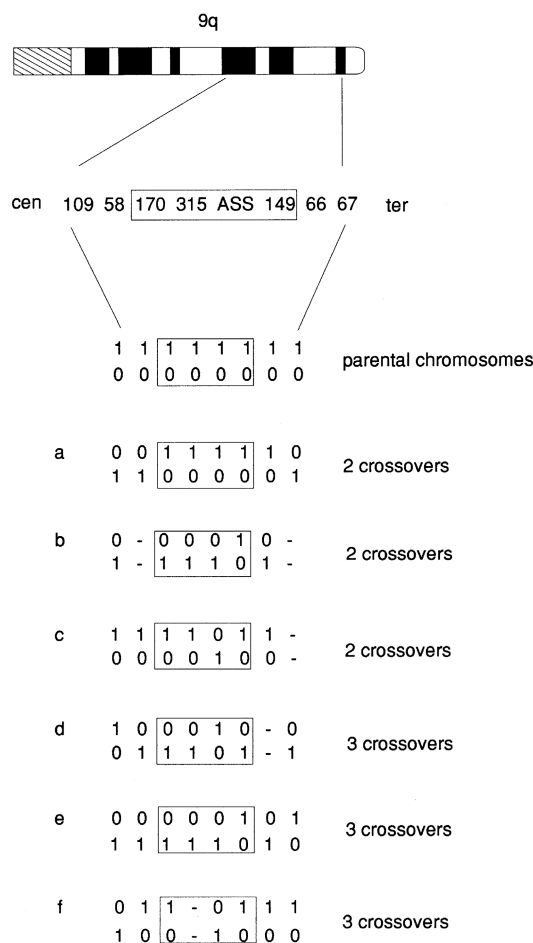


Figure 1 Schematic diagram of six inv(9) recombinant sperm. Each locus is represented by a "0" or "1" indicating the chromosomal origin of the allele. Boxed loci are those within the inversion. Sperm a–f are discussed in the text.

informative for all loci. There were two crossovers: one telomeric to the inversion involving D9S66 and D9S67, the other between D9S58 and the loci within the inversion. All the recombinants of markers within the inversion involve only double recombination events around two markers: either D9S149 or ASS. Sperm b shows a double recombination around D9S149. Sperm c is a double recombinant of ASS. Sperm d had three crossovers, two involving ASS within the inversion. Sperm e and f both had three crossovers—two within the inversion. All of these results were confirmed by retyping the PEP product of each sperm. Interestingly, although double recombinants were evident within the inverted region, there were no single recombination events observed within the inversion.

The data from inv(9) require special consideration for the application of linkage algorithms. Programs such as SPERM and CRI-MAP work on the basic assumption

Table 1**Comparison of Recombinant and Informative Chromosomes of Donors inv(2), Sp9, and inv(9) by Use of CRI-MAP**

INTERVAL NUMBER	MARKER INTERVAL	INV(2)		INV(2) VS. INV(9) P	Sp9		Sp9 VS. INV(9) P	INV(9)	
		R	I		R	I		R	I
1	D9S109–D9S58/59 ^a	26	193	>.05	26	257	>.05	18	208
2	D9S58/59 ^a –D9S154/170 ^b	9	204	>.05	12	269	>.05	15	223
3	D9S154/170 ^b –D9S315	20	211	.0004	26	304	.0005	0	222
4	D9S315–ASS	12	245	.036	15	326	.043	2	180
5	ASS–D9S149	4	256	>.05	5	324	>.05	5	147
6	D9S149–D9S66	11	307	>.05	5	132
7	D9S66–D9S67	22	245	.0022	18	72

NOTE.—R = recombinant chromosomes; I = informative chromosomes.

^a Data use marker D9S59 for donors inv(2) and Sp9 and D9S58 for inv(9).^b Data use marker D9S154 for donors inv(2) and Sp9 and D9S170 for inv(9).

that the order of the markers used is the same on both chromosomes. With inv(9), a subset of these markers—namely, those within the inversion breakpoints—are inverted. This makes linkage analysis problematic. A comparison of the results of inv(9) with donors Sp9 and inv(2) was not possible by use of the SPERM output, since the data involving the crossovers within the inversion were classified as contamination by the algorithm (data not shown). However, a comparison was possible by use of CRI-MAP, which was used to estimate genetic distances and to calculate the number of recombinant and informative chromosomes between adjacent markers. For some loci, a direct comparison of inv(9) with donors inv(2) and Sp9 was not possible owing to lack of informativeness. Since the results obtained with donor inv(2) (above) and those reported elsewhere (Povey et al. 1996) have shown D9S154 and D9S170 to be tightly linked, these markers were treated as a single locus. Similarly, the markers D9S59 (for which Sp9 and inv[2] were informative) and D9S58 (for which inv[9] was informative), which are ~1 cM apart on a male-specific map (Kwiatkowski et al. 1992), were grouped together for subsequent analyses. For the primary analysis, three contiguous regions were considered: region 1, which spanned from the centromeric marker D9S109 to the locus at the centromeric end of the inversion D9S154/D9S170; region 2, within the inversion D9S154/D9S170 to D9S149; and region 3, which spanned from the most telomeric marker of the inversion (D9S149) to D9S67, which is telomeric to the inversion. In region 1, genetic distances were 12.9 cM and 15.3 cM for Sp9 and inv(2), respectively. This compares favorably with 14.9 cM for inv(9). Within the inversion (region 2), distances of 13.6 cM and 20.3 cM were obtained for Sp9 and inv(2), respectively. In inv(9) this distance was reduced to 3.7 cM. Distal to the inversion in region 3, values of 12.9 for Sp9 and 15.5 cM for inv(2) were obtained. For inv(9) the corresponding interval increased to 31.3 cM.

Numbers of recombinant and informative chromo-

somes computed by CRI-MAP for each donor for a given marker interval are summarized in table 1. Analysis of adjacent marker intervals showed differences in recombination between inv(9) and both control donors. No differences were found centromeric to the inversion (intervals 1 and 2), but reduced recombination was evident within the inversion (intervals 3–5), with recombination apparently more suppressed at the centromeric end of the inversion. Thus, in the more centromeric interval 3, no recombinants were observed ($\chi^2 = 18.355$, 1 df, $P < .0001$ for inv[9] vs. Sp9; $\chi^2 = 20.109$, 1 df, $P < .0001$ for inv[9] vs. inv[2]), but 2 recombinants out of 180 informative chromosomes were found in interval 4 ($\chi^2 = 4.109$, 1 df, $P = .043$ for inv[9] vs. Sp9; $\chi^2 = 4.399$, 1 df, $P = .036$ for inv[9] vs. inv[2]) and 5 recombinants out of 147 informative chromosomes in interval 5 ($\chi^2 = .770$, 1 df, $P = .38$ for inv[9] vs. Sp9; $\chi^2 = .674$, 1 df, $P = .42$ for inv[9] vs. inv[2]). The only recombinants within the inversion were double crossovers involving either ASS or D9S149. An increase in recombination was evident in inv(9) in interval 7 ($\chi^2 = 9.357$, 1 df, $P = .0022$ for inv[9] vs. Sp9), but this did not reach significance when the Bonferroni correction for multiple testing was applied (12 independent tests).

Discussion

Interchromosomal Effects

One aspect of this investigation was a study of the potential interchromosomal effect of inv(2) on recombination patterns on other chromosomes, in this instance chromosome 9. No significant differences in recombination were found for intervals common to both Sp9 and inv(2), or between these donors and the CEPH data (not shown). Studies on inversion heterozygotes in other species have reported crossover suppression in the inverted region and increased recombination elsewhere on the same chromosome (Luchessi 1976; Zetka and Rose

1992) and on other chromosomes (Chadov and Chadova 1994). Electron microscopic studies of synaptonemal complexes in human male inversion carriers have also indicated that interchromosomal effects occur in humans (Batanian and Hulten 1987). Overall, donor inv(2) had a longer genetic map than that of the normal control Sp9 (51.1 cM compared with 39.4 cM, respectively). However, there were no significant differences when adjacent intervals were compared, and therefore we conclude the paracentric chromosome 2 inversion had no significant interchromosomal effect on genetic recombination in the region studied on chromosome 9.

Normal Population Variation

The differences in recombination between the normal control Sp9 and inv(2) may be explained by natural variation. In bulls (Park et al. 1995), large variations between chiasma frequency and distribution between individuals have been observed. In humans, there are very few reports of studies of heterogeneity between individuals of the same sex, age, and cultural background. Laurie et al. (1981) carried out a study of the chiasma frequency on three chromosomes for seven males. They found little variation in frequency and distribution of chiasmata. They concluded that, for humans, there may be less variation in chiasma distribution and frequency, for two reasons: first, high rates of recombination coupled with positive chiasma interference may limit the possible sites of chiasma formation. Second, in humans, there may be a greater degree of specific localization of chiasmata—that is, specific sites for crossing over. Nevertheless, this and later studies (Laurie and Hulten 1985a, 1985b) demonstrated substantial variation in recombination in human males with normal karyotypes. Recently, in the first report of statistically substantiated differences between genetic recombination rates in males, Yu et al. (1996) found that, by sperm typing five donors for markers on chromosome 6, statistically significant differences in the recombination fractions were obtained, in the range of 5.1%–11.2%. It was proposed that these differences may be a consequence of polymorphisms in the genes affecting recombination or physical differences in chromosomal structure between individuals.

9q32-q34 Inversion

Patient inv(9) was heterozygous for a paracentric inversion 9q32-q34. Centromeric to the inversion, there were normal levels of recombination when compared with the data of the normal control Sp9 and inv(2). Within the inversion, there was a highly significant reduction in recombination compared with both controls. Telomeric to the distal breakpoint, there was an increase in recombination, from 15.5 cM in Sp9 to 31.3 cM in

inv(9). Analysis of adjacent intervals showed the increase to be localized to interval 7 (between D9S66 and D9S67), which is distal to the inversion. Although comparison of inv(9) to Sp9 in this interval gave a *P* value of .002 (table 1), this did not achieve statistical significance when correcting for multiple testing.

No single recombinants were found with the crossover occurring within the inversion. There were five apparent double recombinants—three involving ASS and two involving D9S149. Apart from these apparent doubles, there is no recombination within the inverted segment. It is possible, but highly improbable, that each double recombinant is the product of contamination, mutation, or PCR error, and in fact there is no recombination across the inverted segment. Contamination of the well with a sperm of the opposite phase and subsequent amplification of seven loci from the original chromosome and only one locus from the contaminating chromosome may have given these haplotypes. Gene conversion events or PCR mutation may also be responsible for these reversals of phase of one of the loci. D9S149 alleles were adjacent in inv(9). The most common mutation is loss or gain of one repeat unit. Alleles for ASS, however, were separated by 10 base pairs. Since there are 5 of these particular recombinants in total out of 270 informative cells, this would seem an unreasonably high mutation rate (1.85×10^{-2}), given previously published levels of $\sim 1 \times 10^{-4}$ (Zahn and Kwiatkowski 1995).

As reported elsewhere in humans, sperm from an individual heterozygous for a paracentric inversion on chromosome 7 showed no chromosomal abnormalities (Martin 1986). The pattern of genetic recombination found for inv(9) would therefore be expected to follow either of two classical mechanisms: if looping of the inverted segment occurred, then the products of an uneven number of recombination events across the inverted region would be expected to be acentric fragments and dicentric anaphase bridges. These would manifest themselves as duplications and deletions of subsets of the markers, depending on the locations of the crossovers within the loop. Duplications would be revealed by both alleles of a marker or series of markers being present in a sperm. Deletions would mean loss of genetic information from one or a series of markers. No such results were found. No patterns of amplification of both alleles were observed in sperm. Similarly, although occasionally some loci failed to amplify, these appeared random, more probably reflecting the efficiency of PCR rather than a pattern of crossing-over behavior. Because of the lack of such products of single recombination events, it would appear that classical inversion loops do not occur at meiosis in donor inv(9). If no chromosome looping occurs, then homologous synapsis would not be achieved. Classically, crossover suppression within the inversion would be expected. This was found, to a large

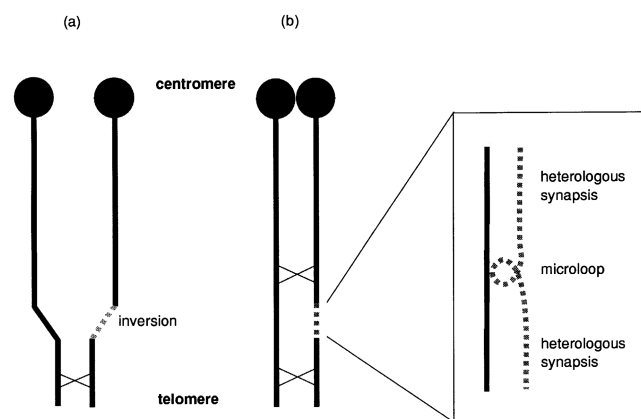


Figure 2 Putative mechanism of recombination within the inverted region of inv(9). Only pairing chromatids are shown, with the inversion segment indicated. See text for a description.

extent. Recombination in the genetic interval bounded by D9S154/D9S170 and D9S149 was 27% of the genetic distance obtained with Sp9 and 18% of the distance obtained with inv(2).

Only double recombination events within the inversion were observed. It is important to point out that single recombination events may occur within this inversion at meiosis, but the products of such recombination may not be found in mature sperm. Sperm cells are the distantly removed products of meiosis and meiotic recombination. For normal development of spermatocytes to occur, complete synapsis of autosomal segments in meiosis I is required. In the case of inversion heterozygotes there is an absence of complete homologous synapsis, and cells are rescued from arresting by heterologous synapsis at a later stage (Saadallah and Hulten 1986). Gametes that receive an abnormal haplotype as a result of meiotic crossing over may never be seen because of the inviability of such chromosomally imbalanced gametes arresting before maturity. That no abnormal sperm were found in these experiments may also be due to the method of sorting of sperm cells. Double recombinants within the heterologously synapsed region would resolve the products into normal chromosomes. Sperm containing these products would mature and would be morphologically normal.

Possible Mechanism of Recombination within 9q32-q34.3 Inverted Region

A putative model of recombination, shown in figure 2, considers that synapsis between chromatids in spermatocytes initiates normally at the telomeres (fig. 2a). As synapsis travels toward the center of the bivalents, it encounters a region of heterosynapsis at the boundaries of the inversion breakpoint. The synapsis stalls at

the region of the telomeric breakpoint. This stalling results in an increase in recombination in the homologously synapsed region. This model is supported by the increase in the subtelomeric region (region 7). Areas of homologous synapsis eventually form centromeric to the inversion and recombination can occur. In the intervening region of nonhomology, an "active search" for homology may force chromatids into forming a structural microloop in the middle of the inversion segment, facilitating rare crossing-over events over a small region of homology (fig. 2b). A novel hot spot of recombination may thus have been created, and only double recombination events involving the same chromatids will resolve into morphologically normal chromosomes. Interestingly, the double recombination events observed in inv(9) sperm are restricted to ASS and D9S149. This may be due to their relatively close physical localization at the midpoint of the inversion and to the nature of the chromatid structure at the time of localized homologous synapsis. This model is supported by the observations of Batanian and Hulten (1987) and Winsor et al. (1978), who studied pericentric inversions of human chromosomes 1 and 9, respectively. Batanian and Hulten (1987) showed that in a large pericentric inversion, initiation of synapsis took place in the middle of the inverted segment with the formation of a microloop. Winsor et al. (1978) found no evidence of abnormal recombinant single crossovers within a small pericentric inversion. In the present study it was not possible to obtain testicular biopsy material from inv(9) for meiotic studies that might have supported our model for recombination.

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